

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
31 October 2002 (31.10.2002)

PCT

(10) International Publication Number
WO 02/086144 A3

(51) International Patent Classification⁷: **C07K 11/00**,
C12N 1/00, 15/74, C07H 21/04

(21) International Application Number: PCT/US02/12331

(22) International Filing Date: 19 April 2002 (19.04.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/284,528 19 April 2001 (19.04.2001) US

(71) Applicant: **INVITROGEN CORPORATION** [US/US];
1600 Faraday Avenue, Carlsbad, CA 92008 (US).

(72) Inventors: **BYRD, Devon, R., N.**; 9511 Briar Glenn Way,
Montgomery Village, MD 20886 (US). **ESPOSITO, Do-**
minic; 6352 Burnt Mountain Path, Columbia, MD 21045
(US).

(74) Agents: **ESMOND, Robert, W.** et al.; Sterne, Kessler,
Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Av-
enue N.W., Washington, DC 20005-3934 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN,
YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:
4 December 2003

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: COMPOSITIONS AND METHODS FOR RECOMBINATIONAL CLONING OF NUCLEIC ACID MOLECULES

(57) Abstract: The present invention relates generally to recombinant DNA technology. The invention relates more specifically to compositions and methods for recombinational cloning of nucleic acid molecules using recombination systems. In particular, the invention relates to compositions comprising one or more Fis proteins and one or more additional components used for recombinational cloning (such as one or more recombination proteins). The invention further relates to the use of the above compositions in methods of recombinational cloning of nucleic acid molecules. The invention also relates to isolated nucleic acid molecules produced by methods of the invention, to vectors comprising such nucleic acid molecules, and to host cells comprising such nucleic acid molecules and vectors.



WO 02/086144 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/12331

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 11/00; C12N 01/00, 15/74; C07H 21/04
 US CL : 435/243, 471; 530/324; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/243, 471; 530/324; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 WEST; DERWENT; BIOSIS; CAPLUS; EMBASE; MEDLINE; WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ✓	Osuna et al. Identification of two functional regions in Fis: The N-terminus is required to promote Hin-mediated DNA inversion but not lambda excision. EMBO Journal, 1991, Vol. 10, No. 6, pages 1593-1603, especially the abstract, figure 3, and materials and methods.	1, 11, 12, 14-23, 26, 29, 34, 44, 45, 47-49, 51-53, 69-71
X ✓	Johnson et al. Isolation of the gene encoding the Hin recombinational enhancer binding protein. PNAS, May 1988, Vol. 85, pages 3484-3488, especially the abstract, materials and methods, figure 3 and Table 2.	1, 11, 12, 14, 15, 18-23, 29, 34, 44, 45, 47-53, 69-71
X ✓	Koch et al. Escherichia coli host factor for site-specific DNA inversion: Cloning and characterization of the Fis gene. PNAS, June 1988, pages 4237-4241, especially the abstract, materials and methods, figure 2 and page 4220, col. 1.	1, 11-15, 18-25, 34, 44-48



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

02 May 2003 (02.05.2003)

Date of mailing of the international search report

23 JUL 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
 Commissioner for Patents
 P.O. Box 1450
 Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer

Remy Yucel

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/12331

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 77 and 78
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 78 depends from itself and claim 77 depends from claim 78, rendering claims 77 and 78 unsearchable.
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-53, 69-76 and 79-90, drawn to a composition comprising a recombinational protein and Fis protein and methods of use.

Group II, claim(s) 54-68, drawn to a method for cloning comprising a nucleic acid segment flanked by two recombination sites which do not recombine with each other.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The method of Group I, claims 35-50, comprises a method of recombination using the composition of claim 1. The method of Group II comprises a nucleic acid flanked by two recombination sites which do not recombine. The method of Group II requires a different means of recombination which is not included in within the scope of the claims of Group I, making the method of Group II distinct from the method of Group I. Therefore, Group I does not share the same technical feature of Group II, and is restrictable into a separate group.